UNCLASSIFIED
SECURITY CLASSIFICATION OF THIS PAGE

DTIC FILE COPY

(2)

REPORT DOCUMENTATION PAGE

AD-A202 977

1b. RESTRICTIVE MARKINGS

3. DISTRIBUTION/AVAILABILITY OF REPORT
Approved for public release; distribution is unlimited

4. PERFORMING ORGANIZATION REPORT NUMBER(S)
NMRI 87-80

5. MONITORING ORGANIZATION REPORT NUMBER(S)

6a. NAME OF PERFORMING ORGANIZATION
Naval Medical Research

6b. OFFICE SYMBOL (If applicable)

7a. NAME OF MONITORING ORGANIZATION
Naval Medical Command

6c. ADDRESS (City, State, and ZIP Code)
Bethesda, Maryland 20814-5055

7b. ADDRESS (City, State, and ZIP Code)
Department of the Navy
Washington, D.C. 20372-5120

8a. NAME OF FUNDING/SPONSORING ORGANIZATION
Naval Medical Research and Development Command

8b. OFFICE SYMBOL (If applicable)

9. PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER

8c. ADDRESS (City, State, and ZIP Code)
Bethesda, Maryland 20814-5055

10. SOURCE OF FUNDING NUMBERS

PROGRAM ELEMENT NO.	PROJECT NO.	TASK NO.	WORK UNIT ACCESSION NO.
63706N	M0095	003.1007	DN677130

11. TITLE (Include Security Classification)
Mls is not a Single Gene, Allelic System

12. PERSONAL AUTHOR(S)
Abe,R.; Ryan, J.J.; Hodes, R.J.

13a. TYPE OF REPORT	13b. TIME COVERED	14. DATE OF REPORT (Year, Month, Day)	15. PAGE COUNT
journal article	FROM ____ TO ____	1987	6

16. SUPPLEMENTARY NOTATION
in: Journal of Experimental Medicine v.166, n.4, Oct.1987, pp.1150-1155

17. COSATI CODES		
FIELD	GROUP	SUB-GROUP

18. SUBJECT TERMS (Continue on reverse if necessary and identify by block number)
Alleles
Antigens, surface
Genes
Mice

19. ABSTRACT (Continue on reverse if necessary and identify by block number)

20. DISTRIBUTION/AVAILABILITY OF ABSTRACT
☒ UNCLASSIFIED/UNLIMITED ☐ SAME AS RPT ☐ DTIC USERS

21. ABSTRACT SECURITY CLASSIFICATION
Unclassified

22a. NAME OF RESPONSIBLE INDIVIDUAL
Phyllis Blum, Information Services Division

22b. TELEPHONE (Include Area Code)
202-295-2188

22c. OFFICE SYMBOL
ISD/ADMIN/NMRI

DD FORM 1473, 84 MAR

83 APR edition may be used until exhausted.
All other editions are obsolete.

SECURITY CLASSIFICATION OF THIS PAGE
UNCLASSIFIED

Brief Definitive Report

Mls IS NOT A SINGLE GENE, ALLELIC SYSTEM

Different Stimulatory Mls Determinants Are the Products of at Least Two Nonallelic, Unlinked Genes

BY RYO ABE,* JOHN J. RYAN,‡ AND RICHARD J. HODES*

*From the *Immunology Branch, National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20892; and the ‡Transplantation Research Program Center, Naval Medical Research Institute, Bethesda, Maryland 20814*

The set of cell surface determinants encoded by the MHC is remarkable in that it is recognized by naive T cell populations at a sufficiently high precursor frequency to induce substantial primary proliferative responses, and the critical biologic role played by MHC products has been well established. The only other determinants capable of inducing strong proliferative responses by naive T cells have been identified in the mouse as minor lymphocyte stimulating (Mls) gene products (1, 2). It was originally proposed by Festenstein that Mls is a single gene system mapped to chromosome 1 (3), and that the Mls locus has at least four alleles, a, b, c, and d (1), which encode polymorphic cell surface structures on B cells and macrophages. Subsequent studies have documented the extraordinarily high frequency of Mls^a- or Mls^d-reactive T cells encountered in both naive (2) and cloned T cell populations (4).

In spite of its initial characterization, the issues of polymorphism and allelism in the Mls system are matters of significant controversy. Because of a high degree of observed crossreactivity between Mls^a and Mls^d determinants (5–7) and the nonexistent or weak stimulatory ability of Mls^b and Mls^c stimulators, some investigators (7) have suggested that the Mls locus has only two alleles, the "a/d" allele encoding nonpolymorphic determinants which cause strong MLR, and the nonstimulatory or null "b" allele. Based on this interpretation, it has recently been proposed that the Mls gene encodes molecules that cause nonspecific mitogenic stimulation to T cells (7) or that Mls products are nonpolymorphic cell interaction structures that are of generalized importance in influencing the interaction between APCs and responding T cells (8). In an attempt to better define polymorphism and allelism in the Mls system, studies were initiated using cloned T cells specific for each of the stimulatory Mls alleles. These studies demonstrated that Mls^a and Mls^c determinants as recognized by cloned T cells are reciprocally non-crossreactive, indicating that polymorphism does indeed exist in the Mls system (9). More strikingly, it was also found that Mls^d strains coexpress both Mls^a-like and Mls^c-like determinants, and that these two sets of determinants are encoded by unlinked genes in the Mls^d genome (10). These

J. J. Ryan was supported by Naval Medical Research and Development Command work unit M0095.001.1055. Address correspondence to Ryo Abe, Bldg. 10, Rm 4B17, NIH, Bethesda, MD 20892.

findings suggested the possibility that the genes encoding Mls^a and Mls^c were themselves nonallelic, and that the original description of Mls^a, Mls^c, and Mls^d as allelic products of a single locus was not correct. To formally analyze the genetic characteristics of the Mls gene system, a segregation analysis of genes encoding Mls^a and Mls^c determinants was carried out.

Materials and Methods

Mice. AKR/J, B10.BR, and C3H/HeJ mice were purchased from The Jackson Laboratory, Bar Harbor, ME. (AKR/J × B10.BR)F_1, (AKR/J × C3H/HeJ)F_1, and (AKR/J × C3H/HeJ) × B10.BR mice were bred in our facilities.

Antibody. Goat anti–mouse IgD antibody was kindly provided by Dr. Fred D. Finkelman (Uniformed Services University of the Health Sciences, Bethesda, MD). The use of this antibody has been described elsewhere (9–11).

T Cell Clones. The generation and characterization of Mls^a-, Mls^c-, and I-A^k-reactive clones have been described (9).

T Cell Proliferation Assay. T cell proliferation was assayed as previously described (9, 10). Results are expressed as the arithmetic means of triplicate cultures. Standard errors were generally <10% of the mean.

Results

Segregation Analysis of Genes Encoding Mls^a and Mls^c Determinants. To evaluate the allelic or nonallelic nature of the genes encoding Mls^a and Mls^c determinants, a segregation analysis was carried out by testing the stimulatory capacity of spleen cells from progeny of the (AKR/J × C3H/HeJ)F_1 × B10.BR [(Mls^a × Mls^c)F_1 × Mls^b] breeding. The hypothetical basis of this segregation analysis was the following. If the Mls^a and Mls^c determinants were encoded by allelic genes, all of the offspring from this breeding would be either $Mls^{a/b}$ or $Mls^{c/b}$ genotypically. Since Mls^b is nonstimulatory, there would be phenotypically only two types of stimulator cells, one of these Mls^a and the other Mls^c. Alternatively, if the genes encoding Mls^a and Mls^c were not allelic, then stimulator cells from some progeny might express both Mls^a and Mls^c or neither Mls^a nor Mls^c. A total of 70 progeny from the (AKR/J × C3H/HeJ)F_1 × B10.BR breeding were tested in five different experiments. In the first experiment presented here, mitomycin C (MMC)-inactivated spleen cells from these progeny were used as stimulators and were cocultured with unprimed C3H/HeJ (Mls^c) nylon wool nonadherent T cells (NNT) to detect Mls^a determinants, with (AKR/J × B10.BR)F_1 ($Mls^{a/b}$) NNT to detect Mls^c determinants, or with cloned I-A^k-specific T cells to test the overall stimulatory abilities of each stimulator cell population (Table I). Spleen cells from all 10 progeny stimulated the I-A^k-specific T cell clone. Stimulator cells from some of these progeny stimulated C3H/HeJ (Mls^c) NNT, but not (AKR/J × B10.BR)F_1 ($Mls^{a/b}$)NNT (Nos. 5, 6), indicating that these cells express Mls^a but not Mls^c (consistent with an $Mls^{a/b}$ genotype); whereas cells from other progeny stimulated only NNT from (AKR/J × B10.BR)F_1, but not C3H/HeJ (Nos. 4, 10), indicating that they expressed Mls^c but not Mls^a (consistent with an $Mls^{c/b}$ genotype). In addition, however, some offspring populations stimulated both C3H/HeJ and (AKR/J × B10.BR)F_1 NNT (Nos. 2, 3, and 8), indicating coexpression of Mls^a and Mls^c by these stimulators; and others stimulated neither of these T cell populations (Nos. 1, 7, and 9), indicating expression of neither Mls^a nor Mls^c.

TABLE I

Primary MLR Induced by Stimulators from (AKR/J × C3H/HeJ)F_1 × B10.B Offspring

Stimulator strain*	Responder T cells‡			Mls type§	
	C3H/HeJ (Anti-Mls^a)	(AKR/J × B10.BR)F_1 (Anti-Mls^c)	BC3C4 (Anti-I-A^k)	a	c
(AKR/J × B10.BR)F_1	78,388‖	15,360	47,538	+	NA
C3H/HeJ	8,211	58,281	83,159	NA	+
(AKR/J × C3H/HeJ)F_1	140,145	51,809	43,808	+	+
(AKR × C3H)F_1 × B10.BR					
1	6,451	16,126	16,708	−	−
2	53,061	38,903	32,253	+	+
3	56,545	31,777	27,855	+	+
4	8,511	26,495	53,877	−	+
5	36,308	15,453	26,189	+	−
6	41,385	15,848	20,015	+	−
7	5,372	17,331	13,413	−	−
8	15,194	29,021	13,476	+	+
9	9,875	17,912	19,105	−	−
10	4,394	22,762	30,727	−	+

* 5 × 10^5 MMC-treated stimulator cells were added to each culture.
‡ 3 × 10^5 unprimed NNT or 10^4 cloned T cells were cultured with the stimulator cells.
§ Typing of Mls was based on the experimental results shown. NA, not appropriate.
‖ Arithmetic mean of [^{3}H]TdR incorporation of triplicate cultures. Underlined numbers are those that are significantly greater ($p < 0.025$ based on the student's t test) than responses to syngeneic stimulators (except responses of the I-A^k-specific clone BC3C4 which are compared to incorporation of clone alone plus incorporation of stimulator alone).

TABLE II

Primary MLR Induced by Stimulators from (AKR/J × C3H/HeJ) × B10.BR Offspring Treated In Vivo with Anti-IgD Antibody

Stimulator strain*	Responder T cells‡			Mls type§	
	C3H/HeJ (Anti-Mls^a)	AKR/J (Anti-Mls^c)	BC3C4 (Anti-I-A^k)	a	c
AKR/J	32,395‖	3,707	261,112	+	NA
C3H/HeJ	14,970	166,609	267,539	NA	+
(AKR/J × C3H/HeJ)F_1	335,783	19,223	293,831	+	+
(AKR × C3H)F_1 × B10.BR					
11	5,539	13,727	296,785	−	+
12	265,438	4,087	212,317	+	−
13	8,234	10,596	252,575	−	+
14	6,540	2,365	275,898	−	−
15	7,732	16,583	262,761	−	+
16	9,167	4,033	227,260	−	−
17	380,220	6,615	246,321	+	+
18	7,301	9,044	254,957	−	+
19	264,086	34,375	181,295	+	+
20	207,580	42,173	107,255	+	+

* Splenic stimulator cells were obtained from mice that were injected with 200 μl goat anti-mouse IgD antiserum 24 h before mice were killed. Cells were inactivated by 3,000 rad irradiation and 5 × 10^5 cells were added to each culture.
‡ 3 × 10^5 unprimed NNT or 10^4 cloned T cells were cultured with the stimulator cells.
§ Typing of Mls was based on the experimental results shown. NA, not appropriate.
‖ See Table I.

This analysis was extended by two additional approaches. In the first of these, the magnitude of primary Mls^c responses was enhanced by using stimulator cells from mice which had been treated in vivo with anti-IgD antibody (11). When assayed for their ability to stimulate NNT from C3H/HeJ (Mls^c) or AKR/J (Mls^a)

TABLE III

Responses of Mls^a- and Mls^c-specific T Cell Clones to Stimulators from (AKR/J × C3H/HeJ) × B10.BR Offspring

Stimulator strain*	Responder T cells‡					Mls type§	
	Anti-Mls^a clones		Anti-Mls^c clones		Anti-I-A^k clone		
	BARC1	BARB12	BC3B13	BC3C13	BC3C4	a	c
AKR/J	40,332‖	124,453	207	93	47,538	+	−
C3H/HeJ	104	99	57,319	117,106	83,159	−	+
(AKR/J × C3H/HeJ)F$_1$	42,167	164,744	21,929	60,925	43,808	+	+
(AKR × C3H)F$_1$ × B10.BR							
21	105	110	1,514	5,733	16,708	−	+
22	57,559	92,643	3,204	15,365	32,253	+	+
23	109	47	2,184	4,732	28,548	−	+
24	37,214	76,007	4,701	22,421	27,855	+	+
25	105	61	7,739	38,037	53,877	−	+
26	32,765	67,770	1,157	3,947	26,189	+	+
27	46,556	81,831	129	291	20,015	+	−
28	84	153	433	406	13,413	−	−
29	21,853	48,097	70	93	10,753	+	−
30	77	71	131	95	19,105	−	−
31	112	60	3,567	7,512	23,476	−	+
32	111	67	4,111	17,540	30,727	−	+

* 5 × 10^5 MMC-treated stimulator cells were added to each culture.
‡ 10^4 cloned T cells were cultured with the stimulator cells.
§ Typing of Mls was based on the experimental results shown.
‖ See Table I.

mice in order to detect Mls^a and Mls^c expression, respectively, (AKR/J × C3H/HeJ)F$_1$ × B10.BR offspring again revealed phenotypically 4 different patterns of Mls expression: Mls^a type (No. 12), Mls^c type (Nos. 11, 13, 15, and 18), Mls$^{a/c}$ type (Nos. 17, 19, and 20), and Mls^a-negative, Mls^c-negative (Nos. 14, 16) (Table II). In another experiment, the Mls type of additional progeny was examined by using Mls^a- and Mls^c-specific T cell clones (Table III) (9). 12 offspring mice were tested and again separated into four groups: Mls^a-type (Nos. 27, 29); Mls^c-type (Nos. 21, 23, 25, 31, and 32); Mls$^{a/c}$-type (Nos. 22, 24, and 26); and Mls^a-negative, Mls^c-negative (Nos. 28, 30). Of the total of 70 progeny tested in all experiments, 37 exhibited either coexpression or nonexpression of both Mls^a and Mls^c determinants, indicating that these two sets of determinants are encoded by distinct and unlinked genes.

Discussion

The issues of allelism and polymorphism are of fundamental importance to an understanding of the biology of the Mls system. If Mls is interpreted, as has conventionally been the case, as a single locus, multi-allelic polymorphic system, then attempts to understand the nature of T cell recognition of Mls may center on mechanisms involving a polymorphic T cell recognition structure (such as the conventional T cell receptor α/β dimer). In contrast, if the character of Mls products is found to differ from this conventional model, then the nature of the relevant TCR structures for Mls may also differ. We have recently demonstrated (9) that Mls^a-specific clones respond to Mls^a but not Mls^c, while Mls^c-specific clones respond to Mls^c but not Mls^a, a reciprocal pattern of specificity indicating that polymorphism does exist between Mls^a and Mls^c. Surprisingly, however, it

was also found (10) that both Mls^a-specific and Mls^c-specific clones were responsive to Mls^d strain stimulators, indicating that Mls^d strains expressed Mls^a-like as well as Mls^c-like determinants. A genetic analysis further demonstrated that the Mls^a-like and Mls^c-like determinants expressed by Mls^d cells were encoded by nonallelic genes (10). These studies raised the possibility that the prototypic Mls^a and Mls^c products themselves, which were initially described as allelic products of a single locus, might be encoded by independent gene loci. The present study, using monospecific T cell clones to Mls^a and Mls^c, in combination with primary anti-Mls responses, has indicated by formal segregation analysis that the genes encoding Mls^a and Mls^c determinants are neither allelic nor closely linked. In association with recent findings that indicated that anti-Mls^d T cell responses are in fact composed of clonally distinct responses to antigenically noncrossreactive Mls^a and Mls^c determinants (10, 12), the present results suggest that Mls^d does not represent an independent genotype, but rather the coexpression in Mls^d strains of the products of unlinked Mls^a and Mls^c genes.

The findings presented here provide a genetic characterization of the Mls system that is substantially different from the conventional model. What were originally defined as Mls-specific T cell responses now appear to consist of responses to determinants controlled by at least two independent gene loci. One set of these determinants is controlled by a gene located on the Mls locus mapped to chromosome 1, and this gene product (originally designated Mls^a) is expressed on Mls^a- and Mls^d-type cells. Another, controlled by a gene that has not yet been mapped and the products of which have been designated Mls^c, is expressed on Mls^c- and Mls^d-type cells. Since Mls^b is nonstimulatory for T cells in most response systems, there may be no polymorphism within either the Mls^a or the Mls^c system. Because Mls appears to consist of relatively or absolutely nonpolymorphic products of each of two (or more) unlinked genes, the strong stimulatory capacity encoded by Mls genes may be mediated by interactions with T cell structures that are themselves nonpolymorphic, consistent with the previous proposals of Webb et al. (7) and Janeway et al. (8). It remains to be determined whether the determinants encoded by Mls^a and Mls^c are distinct functionally as well as genetically and what role they play in the generation and expression of the antigen-specific T cell repertoire.

Summary

Major Histocompatibility Complex

Mls determinants share with (MHC) products the unique property of stimulating T cells at extraordinarily high precursor frequencies. The Mls system was originally described as a single locus on chromosome 1, with four alleles, Mls^a, Mls^b, Mls^c, and Mls^d, that encode polymorphic cell surface structures. However, the fundamental issues of polymorphism and allelism in the Mls system remain controversial. To clarify these questions, a formal segregation analysis of the genes encoding Mls^a and Mls^c determinants was carried out by testing the capacity of spleen cells from progeny of (Mls^a × Mls^c)F$_1$ × Mls^b breedings to stimulate responses by unprimed T cells and by Mls^a and Mls^c specific cloned T cells. The results of this analysis indicated that the gene encoding Mls^a determinants is neither allelic to nor linked to the gene encoding Mls^c determinants. Together with previous findings (12), these results also suggest that another strongly

stimulatory type, Mls^d, in fact results from the independent expression of unlinked Mls^a and Mls^c gene products. Based on these observations, it is concluded that, contrary to conventional concepts, the stimulatory phenotypes designated as Mls^a, Mls^c, and Mls^d can be accounted for by the independent expression of the products of at least two unlinked gene loci.

The authors wish to thank Drs. R. E. Gress and D. H. Sachs for their critical reviews of this manuscript. We thank Mr. F. Jones and Ms. L. DeNenno for their care of the experimental animals.

Received for publication 1 June 1987 and in revised form 6 July 1987.

References

1. Festenstein, H. 1973. Immunogenetic and biological aspects of in vitro lymphocyte allotransformation (MLR) in the mouse. *Transplant Rev.* 15:62.
2. Janeway, C. A., Jr., E. A. Lerner, J. M. Jason, and B. Jones. 1980. T lymphocytes responding to Mls-locus antigens are Lyt-1$^+$,2$^-$ and I-A restricted. *Immunogenetics.* 10:481.
3. Festenstein, H., C. Bishop, and B. A. Taylor. 1977. Location of Mls locus on mouse chromosome 1. *Immunogenetics.* 5:357.
4. Lynch, D. H., R. E. Gress, B. W. Needleman, S. A. Rosenberg, and R. J. Hodes. 1985. T cell response to Mls determinants are restricted by crossreactive MHC determinants. *J. Immunol.* 134:2071.
5. Ryan, J. J., A. Ahmed, P. Kind, and K. W. Sell. 1979. Crossreactivity exists between Mls^a and Mls^d lymphocyte-activating determinants as demonstrated by the negative clonal selection of responder cells in a mixed lymphocyte reaction. *Transplant. Proc.* 9:1377.
6. Molnar-Kimber, K. L., and J. Sprent. 1981. Evidence that strong Mls determinants are nonpolymorphic. *Transplantation (Baltimore).* 31:376.
7. Webb, S. R., K. Molnar-Kimber, B. Jennifer, J. Sprent, and D. B. Wilson. 1981. T cell clones with dual specificity for Mls and various major histocompatibility complex determinants. *J. Exp. Med.* 154:1970.
8. Janeway, C. A., Jr., P. J. Conrad, J. Tite, B. Jones, and D. B. Murphy. 1983. Efficiency of antigen presentation differs in mice differing at the Mls-locus. *Nature (Lond.).* 306:80.
9. Abe, R., J. J. Ryan, F. D. Finkelman, and R. J. Hodes. 1987. T cell recognition of Mls: T cell clones demonstrate polymorphism between Mls^a, Mls^c and Mls^d. *J. Immunol.* 138:373.
10. Abe, R., J. J. Ryan, and R. J. Hodes. 1987. Clonal analysis of the Mls system. A reappraisal of polymorphism and allelism among Mls^a, Mls^c, and Mls^d. *J. Exp. Med.* 165:1113.
11. Ryan, J. J., J. J. Mond, F. D. Finkelman, and I. Scher. 1983. Enhancement of the mixed lymphocyte reaction by in vivo treatment of stimulator spleen cells with anti-IgD antibody. *J. Immunol.* 130:2534.
12. Ryan, J. J., J. J. Mond, and F. D. Finkelman. 1987. The Mls^d-defined primary mixed lymphocyte reaction: a composite response to Mls^a and Mls^c determinants. *J. Immunol.* 138:4085.